

This listing of claims will replace all prior versions of claims in the application.

Listing of Claims: Please amend the claims as follows:

We claim:

Claim 1. (Currently Amended) ~~Variant A~~ A polypeptide variant of the major allergen Phl p 1 from timothy grass, ~~characterised in that it has which comprises~~ an additional Cys residue compared with to the wild type Phl p 1 sequence, wherein the location of said additional Cys residue is higher than amino acid position 230 of said wild type Phl p 1 sequence.

Claim 2. (Cancelled)

Claim 3. (Cancelled)

Claim 4. (Currently Amended) ~~Allergen~~ The polypeptide variant according to claim 1, ~~characterised in that wherein~~ the additional Cys residue is located between amino acid positions 230 and 240 of said wild-type Phl p 1 sequence.

Claim 5. (Currently Amended) ~~Allergen~~ The polypeptide variant according to claim 1, ~~characterised in that wherein~~ the additional Cys residue originates from an amino acid exchange.

Claim 6. (Currently Amended) ~~Allergen~~ The polypeptide variant rPhl p 1-A236C ~~according to SEQ ID NO 2 according to claim 1~~ which comprises a polypeptide sequence set forth in SEQ ID NO: 2, characterised in that wherein the additional Cys residue has been introduced by exchange of Ala 236.

Claim 7. (Withdrawn) DNA molecule which encodes for an allergen variant according to claim 1.

Claim 8. (Withdrawn) DNA molecule according to SEQ ID NO 1 which encodes for the allergen variant according to Claim 6.

Claim 9. (Withdrawn, Currently Amended) ~~Process A~~ a process for the preparation of a polypeptide variant of the recombinant major allergen rPhl p 1 according to claim 1 comprising:

characterised in that, by methods known per se,

- [[]] (a) introducing a base triplet encoding for which encodes a Cys residue is introduced the corresponding gene into a polynucleotide encoding said rPhl p 1 by insertion or exchange, wherein said additional Cys residue is located in a higher position than amino acid position 230 compared with the wild type Phl p 1 polypeptide sequence;
- [[]] (b) introducing the gene modified in this way is overexpressed in said polynucleotide of (a) into a host organism and culturing said host organism under sufficient conditions to allow expression of said polypeptide variant; and
- [[]] (c) purifying the allergen polypeptide variant obtained by overexpression is purified.

Claim 10. (Withdrawn, Currently Amended) Process A process for the preparation and purification of a variant of the recombinant major allergen rPhl p 1 according to Claim 9 in soluble form, characterised in that the initially comprising
obtaining an insoluble crude protein according to claim 9,
denaturing said insoluble crude protein
and
further renaturing said denatured crude protein is denatured, subsequently renatured by dilution and purified by biochemical purification steps.

Claim 11. (Withdrawn, Currently Amended) Process A process for the purification of a variant of the recombinant major allergen rPhl p 1 according to Claim 9 in soluble form, characterised in that, starting from the overexpressed, initially insoluble crude protein provided with an His-tag for purification purposes, a plurality of biochemical purification steps, encompassing comprising
employing a polynucleotide which encodes a fusion protein comprising a His-tag and said polypeptide variant of major birch allergen rPhl p 1,
expressing said His-tagged polypeptide in said host organism,
purifying said His-tagged polypeptide using two-stage metal ion chelate affinity chromatography
and the removal of the
removing said His tag to obtain said polypeptide variant of major birch allergen rPhl p 1 in soluble form, are carried out.

Claim 12. (Currently Amended) ~~Allergen variant according to claim 1, characterised in that it~~
The polypeptide variant of claim 1 which exists in various fold forms.

Claim 13. (Currently Amended) ~~Fold A fold form rPhl p 1-LM of the allergen polypeptide~~
variant according to claim 1, which is obtainable by ~~carrying out the following process steps:~~

- ~~[[--]]~~ (a) ~~overexpression of the overexpressing in a host organism, a fusion protein comprising~~
the rPhl p 1 allergen polypeptide variant provided with an and a His tag in a host
organism;
- ~~[[--]]~~ (b) ~~denaturing of the inclusion bodies isolated from the host organism using guanidinium~~
chloride;
- ~~[[--]]~~ (c) ~~renaturing of the dissolved protein on a chelate affinity chromatography column;~~
- ~~[[--]]~~ (d) ~~removal of removing the His tag;~~
- ~~[[--]]~~ (e) employing gel filtration;
- ~~[[--]]~~ (f) further purifying using chelate affinity chromatography;
- ~~[[--]]~~ (g) ~~isolation of isolating the target protein from the flow-through; and~~
- ~~[[--]]~~ (h) further employing gel filtration.

Claim 14. (Currently Amended) ~~Fold A fold form rPhl p 1-HM of the allergen variant~~
according to claim 1, which is obtainable by ~~carrying out the following process steps:~~

- ~~[[--]]~~ (a) ~~overexpression of the overexpressing in a host organism, a fusion protein comprising~~
the rPhl p 1 allergen polypeptide variant provided with an and a His tag in a host
organism;
- ~~[[--]]~~ (b) ~~denaturing of the inclusion bodies isolated from the host organism using guanidinium~~
chloride;
- ~~[[--]]~~ (c) ~~renaturing of the dissolved protein on a chelate affinity chromatography column;~~
- ~~[[--]]~~ (d) ~~removal of removing the His tag;~~
- ~~[[--]]~~ (e) employing gel filtration;
- ~~[[--]]~~ (f) further purifying using chelate affinity chromatography;
- ~~[[--]]~~ (g) ~~elution of eluting the target protein with an imidazole gradient; and~~
- ~~[[--]]~~ (h) further employing gel filtration.

Claim 15. (Currently Amended) ~~Allergen A vaccine which comprises the polypeptide~~
variant according to claim 1 and an acceptable carrier as medicament.

Claim 16. (Withdrawn, Currently Amended) Use of an allergen variant according to Claim 15 and/or pharmaceutically usable derivatives thereof, including mixtures thereof in all ratios, for the preparation of a medicament A method for specific immunotherapy of allergies in the triggering of which the an allergy triggered by major allergen Phl p 1 from timothy grass comprising administering to a subject in need thereof a polypeptide variant of claim 1 or a pharmaceutical composition thereof is involved.

Claim 17. (Currently Amended) Pharmaceutical A pharmaceutical composition comprising an allergen a polypeptide variant according to Claim 15 and/or pharmaceutically usable derivatives thereof, including mixtures thereof in all ratios, and if desired, excipients and/or adjuvants claim 1 and a pharmaceutically acceptable carrier.

Claim 18. (Withdrawn, Currently Amended) Use of an allergen variant according to claim 1 and/or derivatives thereof, including mixtures thereof in all ratios, A method for the *in vitro* diagnosis of allergies in the triggering of which the an allergy which is triggered by major allergen Phl p 1 from timothy grass comprising administering to a subject in need thereof a polypeptide variant of claim 1 or a pharmaceutical composition thereof is involved.

Claim 19. (Withdrawn) Recombinant DNA expression vector containing a DNA molecule according to Claim 7 for the treatment of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved, by immunotherapeutic DNA vaccination.

Claim 20. (Withdrawn, Currently Amended) Use of the expression vector according to Claim 19 and/or derivatives thereof, including mixtures thereof in all ratios, for the preparation of a medicament A method for the treatment of allergies in the triggering of which the an allergy triggered by major allergen Phl p 1 from timothy grass comprising administering to a subject in need thereof an is involved; by immunotherapeutic DNA vaccination vaccine of claim 15.

Claim 21. (Withdrawn) Pharmaceutical composition comprising an expression vector according to Claim 19 and/or pharmaceutically usable derivatives thereof, including mixtures thereof in all ratios, and, if desired, excipients and/or adjuvants, for the treatment of allergies in the triggering of which the major allergen Phl p 1 from timothy grass is involved,

by immunotherapeutic DNA vaccination.

Claim 22. (New) A polypeptide variant of major allergen Phl p 1 from timothy grass which comprises an additional Cys residue compared to the wild type Phl p 1 sequence, wherein the location of said additional Cys residue is higher than amino acid position 140 of said wild-type sequence.

Claim 23. (New) The polypeptide variant of major allergen Phl p 1 from timothy grass according to claim 22 which is

(a) a polypeptide which is encoded by a polynucleotide comprising the sequence set forth in SEQ ID NO: 1; or

(b) a polypeptide which comprises the sequence set forth in SEQ ID NO: 2.